

The University of Chicago

A Study of Vasectomy on Rats and Guinea Pigs

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF
SCIENCE IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

BY

ROBERT M. OSLUND

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. 67, No. 2
January, 1924



A STUDY OF VASECTOMY ON RATS AND GUINEA PIGS¹

ROBERT OSLUND

From the Department of Zoölogy, University of Chicago

Received for publication September 18, 1923

This investigation began as an attempt to make an analysis of the changes in the testis following vasectomy. Leon Gosselin (1853) introduced the experimental study of effects of ligation and resection of the vas deferens using dogs as experimental material. He found that there was normal spermatogenesis present four to six months after such operation. Brissaud (1880) found the same situation in rabbits. Ancel and Bouin (1903), using rabbits and pigs, secured exactly the opposite results. They stated (p. 499) that the testicles they had experimentally transformed resembled in all respects the testicles of cryptorchid animals, i.e., with seminal tubules reduced and having a single layer of columnar Sertoli cells prolonged into filamentous processes, and with no sperm-forming cells present. Shattock and Seligman (1904), studying sheep, obtained results contrary to those of Ancel and Bouin and stated (p. 52) that "microscopic section of the testis of the normal adult Herdwick sheep and of that from the vasectomized animal of the same age and killed at the same date, show similar histological pictures." Later investigators, although performing the same operation upon rats, guinea pigs and rabbits, have obtained conflicting results. Thus Steinach (1921), Sand (1921) and Lipschutz (1922) have reported degenerative changes followed by regeneration. The last named states that in some animals one testis may undergo degeneration while the other testis remains normal. Sand, on the other hand, finds in many experiments that degeneration does not occur. These are but a few of the workers in this field, but further references would only serve to extend the contradictions already seen. It is evident from these instances that the effects upon the testes of vasectomy operations are not always the same, either because of anatomical and physiological differences in the various animals used, or because of slight variations in the operative technique. In some instances there have also been made inadequate analyses of the situations encountered.

¹ A dissertation submitted to the faculty of the Ogden School of Science in candidacy for the degree of Doctor of Philosophy.

Since there are so many discrepancies in accounts of these operations, it was decided to repeat the experiments on a large scale: constant results were finally obtained and it is the purpose of this paper to give a detailed account of the operative procedures involved, and a report of the findings with analysis of them. It became evident during the early part of this work that the discrepancies in the results obtained were due to a confusion of results of vasectomy and of artificial cryptorchidism. It was found that ligation and resection of the vas deferens does not of itself produce degeneration of the germinal epithelium, and that when such degeneration followed vasectomy it was due to the fact that the testes remained in the abdominal cavity following the operation: such a displacement of itself (artificial cryptorchidism) is sufficient to produce degeneration of the germinal epithelium without vasectomy.

MATERIAL AND TECHNIQUE. Because of the fact that most of the preceding work was done on rats and guinea pigs and because of the availability of these animals they have served as the experimental material of this paper. The rats are of the Wistar Institute strain, of which there is a large, well-kept colony at this laboratory. The guinea pigs are from a local colony of healthy animals. The caretaker knew no distinction in the stock and gave all animals the same care. That the diet was suitable was judged from the health of the colony and from the large size of the litters raised. Controls were from the same groups, often blood brothers, and killed at the same age as the experimental animals.

All operations were performed with aseptic precautions. The animals were etherized, the abdomen clipped of hair, shaved and then washed with an alcoholic solution of iodine. Laparotomy, along the mid-ventral line, was then performed exposing the vasa deferentia, internal spermatic arteries and fat-bodies attached to the testes. In some animals the testes were found in the abdominal cavity, as is common in rodents, while in most cases they were located in the scrotum. At the conclusion of the operation the incision was closed by a continuous suture first of the muscles and then of the skin.

In order to cover the field comprehensively the following modifications of the general procedure were carried out: 1, unilateral vasectomy; 2, bilateral vasectomy; 3, unilateral vasectomy with the contralateral testis removed; 4, bilateral vasectomy with both testes in the scrotum; 5, bilateral vasectomy with one testis in the scrotum, the other testis retained in the abdominal cavity; 6, unilateral vasectomy with the contralateral testis retained in the abdominal cavity; 7, artificial cryptorchidism; and 8, partial castration. Rats ranging in age from thirty days to senescent animals and guinea pigs from twelve days to adults of

more than one year have been used; totaling over two hundred animals.² (Only a representative portion of the experiments is here given.) The animals were killed after periods of from fourteen days to ten months from the time of the operation. Gross peculiarities were noted at that time and the condition of the vas deferens—whether or not it had regenerated—was determined. During the later part of the work the importance of the location of the testes became known. Special attention was then devoted to controlling the place of their residence and in recording the same.

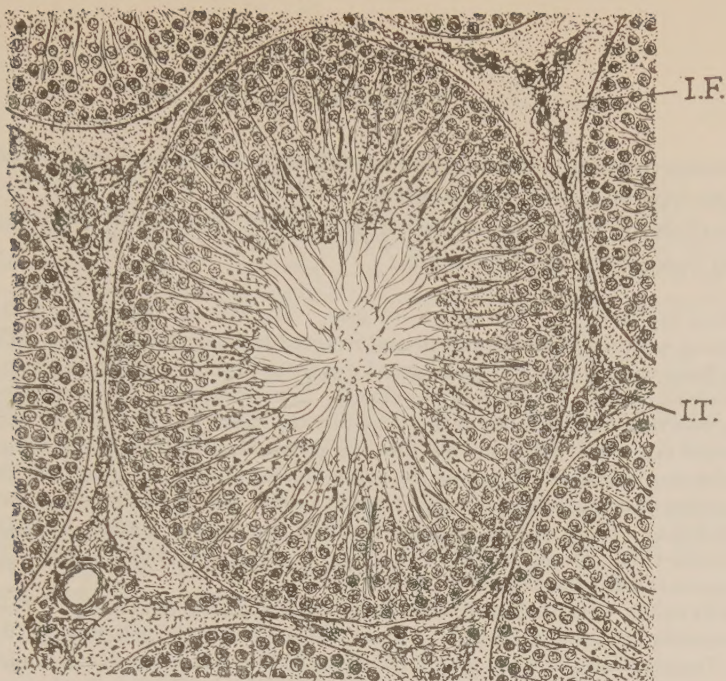
In the preparation of the tissue for study, Allen's (1919 a) injection method of fixation was used. In most cases his modification of Bouin's fluid was employed as the fixing agent, but as the work progressed Bouin's fluid and Formol-Zenker were tried. No important variations were noted in the results from these fixatives that might be attributed to them. The tissues were dehydrated and embedded in paraffin in the manner described by Allen (1919 a) and sections were cut 7 microns in thickness. Many combinations of stains were tried and from each experiment a set of slides stained with Mallory's triple, Harris' hematoxylin and eosin and Delafield's hematoxylin and congo-red were prepared. All comparative observations were made upon representative sections taken from corresponding regions of the different testes.

Although microscopic observations were most relied upon, the condition of the testis could in most cases be recognized by the naked eye, both before and after sectioning. In cases of degeneration the testis was smaller, the tubules contracted and almost devoid of the content. The contrast in microscopic section is plainly seen in figures 1 and 2 drawn to the same scale and of the same animal.

EXPERIMENTAL DATA. For convenience of presentation the experiments have been divided into two groups which, in general, indicate the progress of the investigation. Each group has been subdivided according to the type of operation performed and the results obtained. The experiments of the first group presented were performed before the importance of the position of the testis was suspected. In these experiments, therefore, the location of the testis was seldom noted. The second group comprises those experiments performed during the later part of the work when the variable factor (location of the testis) was controlled.

Group I. Location of the testis not known. A. Degeneration of the germinal epithelium was found to follow unilateral vasectomy in some

² This study was taken up at the suggestion of Prof. F. R. Lillie and is the outgrowth of a series of experiments done at the University of Chicago by Dr. C. R. Moore. I wish to acknowledge my indebtedness to them for interesting me in this problem.



All figures are from camera lucida drawings by Jose K. Santos

Fig. 1. Portion of right testis from rat 38 no. 36. *IF*, intertubular fluid. *IT*, intertubular tissue—interstitial and connective tissue.

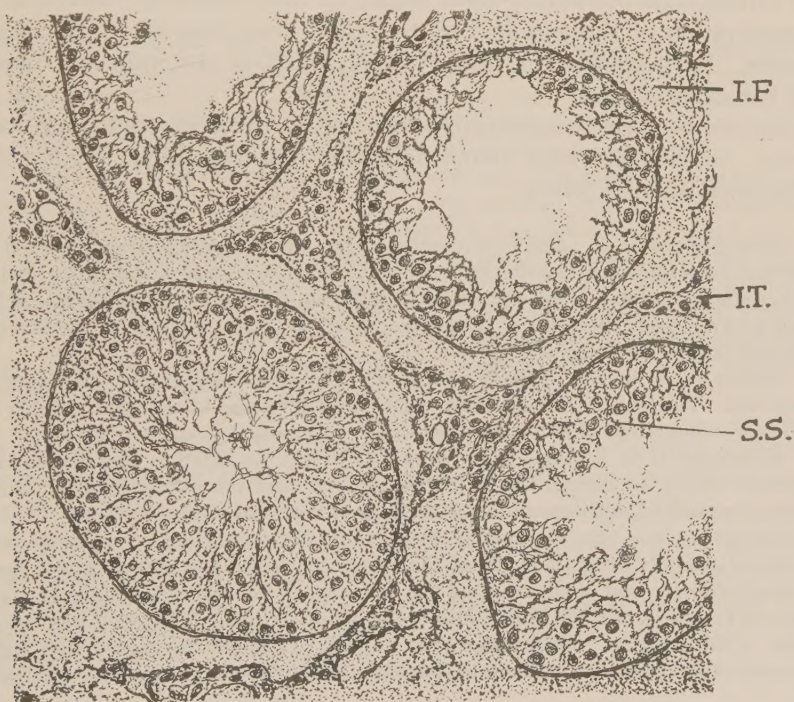


Fig. 2. Portion of left testis from rat 38 no. 36. *IF*, intertubular fluid; *IT*, intertubular tissue; *SS*, syncytium of supporting epithelial cells.

of the experiments, while in others no changes could be found. Examples of each type of results will be given.

Protocols of two cases where degeneration was found to follow unilateral vasectomy are here given:

Guinea pig 40 no. 1. Age about one year. The left vas deferens was ligated in two places with a coarse silk thread and resected between the ligatures. The testis of which the vas deferens had been ligated was then allowed to return of its own accord to the scrotum while the incision was closed. Six months later the animal was killed and at that time the vas deferens was closed by the ligature. The microscopic picture of the left (operated) testis showed that the germinal epithelium had undergone considerable degeneration. Many of the seminiferous tubules were entirely devoid of spermatogenic cells, many had very few active cells while the remaining tubules were full of debris. There was no modification from the average normal condition found in the inter-tubular tissue, either in quantity or in appearance of the cells. The right (unoperated) testis was normal in all respects.

Rat 24 no. 2. Operated upon when three months, eleven days of age. The left vas deferens was ligated and resected, a piece about one-fourth inch in length being cut out. Two months later when the animal was killed the vas deferens was found to have grown together and the lumen of the same was complete. Microscopic sections showed that many of the seminiferous tubules were almost devoid of active cells, while adjoining tubules were full and appeared entirely normal.

Were these, and a few others that might be selected, the total number of experiments performed, there would be little reason for doubting that degeneration of the germinal tissue results from ligation of the vas deferens. Such results are, however, not found in the immense majority of the experiments.

In another selected group of experiments involving unilateral vasectomy no changes were found following the operation. Protocols of four such animals follow:

Guinea pig 25 no. 5. Age, four months. The right vas deferens was resected only, the testicular end opening into the peritoneal cavity, the urethral end being ligatured. When the animal was killed eight months later the testicular end of the vas deferens had grown closed. Sections of the right (operated) testis, as those of the left testis, showed normal tissue throughout.

Guinea pig 5 no. 27. Age, twelve days. The left vas deferens was ligated and resected. The ligature was still firm when the animal was killed eight months later. Microscopic sections of both testes were alike and normal in every respect.

Guinea pig 11 no. 9. Age, thirty days. The right vas deferens was ligated and resected. When the animal was killed ten months later the vas deferens appeared to have regenerated. The microscopic sections of both testes were normal.

Rat 23 no. 1. Age, ninety-four days. The left vas deferens was resected, the urethral end only being tied. When the animal was killed one month later a cyst had formed over the end of the resected vas deferens. This cyst was about the size of a normal rat testis, and filled the mouth of the inguinal canal. The testis was below the cyst in the scrotum. Both testes were normal. It might be argued that the cyst formed an escape for the products of the seminiferous tubules and that the testis should

not, therefore, be looked upon as having the vas deferens closed. But many cases were found where most of the germinal epithelium had degenerated in the testis, in spite of the presence of a cyst on the vas deferens; for example, in rat 51 no. 23 (see below). Again it might be said that two months was not time enough for the degeneration to have taken place. However, in rat 24 no. 2 given above, many of the seminiferous tubules were almost devoid of active cells one month after the operation.

In none of these cases has there resulted any change in the tissues following ligation of the vas deferens. Yet the procedure was supposedly the same as in those cases given above where degenerative changes were found. In none of these experiments was there any effort made to determine the location of the testis during the period following the operation, until the animal was killed. The inguinal canal was open and the gubernaculum remained attached and it was therefore assumed that the testes would take their normal position when the wound was closed.

B. When the experiments involving bilateral vasectomy were checked over, three classes of results were found, viz: 1, those in which both testes showed degenerative changes; 2, those in which neither testis showed any degenerative changes; and 3, those in which degenerative changes were found in one testis, the other testis being normal. Each of these groups will be taken up separately.

Protocols of animals in which degeneration took place in both testes following bilateral vasectomy will be given first:

Guinea pig 31 no. 36. Age, four months. Both vasa deferentia were ligated and resected. The animal was killed one month later at which time both ligatures on the vasa deferentia were still firm. Microscopic sections showed that degeneration had taken place in most of the tubules of both testes. This degeneration was more evident in the right testis than in the left where many of the tubules were normal.

Rat 51 no. 23. Over one and one-half years of age. The testes appeared normal at the time both vasa deferentia were ligated and resected. The rat was killed four months later at which time a large cyst was found to have grown over the end of each vas deferens and adhesions were holding the entire testicular mass of each side in the abdominal cavity. Sections of each testis showed many seminiferous tubules entirely devoid of spermatogenetic cells, while other tubules appeared normal and full of active cells. This experiment was referred to in a former group as one in which degenerative changes were found to have taken place though a large cyst (in this case each cyst was larger than a normal rat testis) closed the end of the vasa deferentia.

In this group where both vasa deferentia were ligated, there followed degenerative changes, though in one case one testis had undergone more degeneration than the other testis of the same animal. These results are, in general, in agreement with statement of some previous workers, though they are not uniform and therefore somewhat confusing.

In another group of experiments involving bilateral vasectomy the results were of the opposite nature; no degeneration took place. The operations were performed in the same manner as in those experiments

just cited and at the same time. Protocols of four such cases will be given:

Guinea pig 37 no. 6. Age, eight months. Both vasa deferentia were ligated and resected. The animal was killed six months later at which time both ligatures were found to be firm. Microscopic sections of each testis were those of normal testes. No degeneration was found.

Guinea pig 29 no. 34. Age, four months. Both vasa deferentia were ligated and resected. When the animal was killed six months later both vasa deferentia were still closed by the ligatures. Microscopic observations indicated that both testes were normal and free from degenerative changes.

Guinea pig 21 no. 26. Age, two months. Both vasa deferentia were ligated and resected. When the animal was killed two months later, the ligatures were still firm. No changes were found to have taken place in either testis. In none of these three cases were there any cysts present complicating the findings. In guinea pigs 37 no. 6 and 29 no. 34, though the vasa deferentia were ligated during a period of six months, no degeneration followed.

Rat 35 no. 31. Adult. Both vasa deferentia were ligated and resected. When the animal was killed six months later the left vas deferens was closed, but the right vas deferens may have regenerated. This could not be ascertained. Microscopic sections showed that both testes were normal.

In still another group of experiments involving bilateral vasectomy another type of result was found. One testis underwent degeneration while the other testis remained normal. Examples of such experiments follow:

Guinea pig 38 no. 7. Age, eight months. Both vasa deferentia were ligated and resected for a period of six months. Microscopic sections showed that the left testis only had undergone degeneration; the right testis was normal.

Guinea pig 2 no. 12. Age, 12 days. Ligation of both vasa deferentia was complete for a period of ten months. Microscopic observation showed that the germinal epithelium of the right testis only had undergone degeneration; the left testis was normal.

Guinea pig 13 no. 19. Age, thirty days. Though the vasa deferentia were ligated for a period of ten months, the left testis did not undergo degenerative changes. There was some degeneration found in the right testis.

Guinea pig 27 no. 32. Age, four months. Both vasa deferentia were ligated for a period of six months. Degenerative changes were found in the tubules of the right testis while the left testis was normal.

Rat 36 no. 34. Adult. Both vasa deferentia were ligated for a period of six months. When the animal was killed a small adhesion was holding the right testis at the mouth of the inguinal canal. Degenerative changes were found in the right testis only.

In these five experiments the ligation had been for periods of six and ten months. In each case degeneration took place in one testis only, while the other testis remained unaffected by the operation. Since the differences here seen are within the individual animals (the testis of one side as opposed to that of the other side), it cannot be said to be due to the physiological condition of the animals.

By way of summarizing the results of the five groups of experiments we find:

1. Unilateral vasectomy, followed by degeneration of the germinal epithelium.
2. Unilateral vasectomy, followed by no degenerative changes.
3. Bilateral vasectomy, followed by degeneration of germinal epithelium in both testes.
4. Bilateral vasectomy, followed by no degenerative changes in either testis.
5. Bilateral vasectomy, followed by degeneration of the germinal epithelium in one testis only, the other testis remaining normal.

These experiments were all performed as nearly alike as was possible and the dates of operation overlap so that no improvement in technique entered into account. The results were inconstant, and especially in the last group there is a marked irregularity. Time cannot be a causal factor in these experiments since degeneration in one case (guinea pig 31 no. 36) occurred in one month, and in other cases did not occur in ten months.

Group II. Location of the testis known. It is obvious that factors not yet accounted for cause or prevent the degenerative process. It was with the hope of isolating each influence, that many more operations were performed and the technique carefully checked. A clew to the situation had been found in experiments guinea pig 24 no. 4 and in rats 51 no. 23 and 36 no. 34 previously cited, where it was noted that in each case the testis that had undergone degeneration was retained in the abdominal cavity by an adhesion. As has been stated, no attention was devoted in the preceding experiments to determining the location or the condition of the testis between the date of operation and that of death. No definite reference to the location of the testis after vasectomy operations occurs in the literature as far as the writer is aware. Accordingly, a series of operations involving bilateral vasectomy was performed, using the same technique as before, but observing, during the post-operative period, the location of the testes. In some of the experiments of this series the testes were in the scrotum following ligation of the vasa deferentia while in other experiments they were retained in the body cavity. Each type of operation will be considered separately.

A. In one group of animals both testes were known to reside in the scrotum following the vasectomy operation until the animals were killed. Protocols of such cases follow:

Guinea pig 20 no. 25. Adult. Both vasa deferentia were ligated and resected. When the animal was killed forty-five days later the ligatures on the vasa deferentia were firm. The testes were in the scrotum and their location had been ascertained throughout the post-operative period by palpation. Microscopic observations showed that no degeneration had taken place in either testis.

Guinea pig 21 no. 26. Age, two months. Both vasa deferentia were ligated and resected. During the post-operative period of two months, the location of the testes was ascertained to be in the scrotum by palpation from time to time. Neither testis showed any degenerative changes.

Guinea pig 23 no. 54. Age, two months. Both vasa deferentia were ligated and resected. During the three months following it was ascertained, by palpation, that the testes were in the scrotum. Both testes were found to be normal in all respects.

Rat 39 no. 37. Adult. The vasa deferentia were ligated and resected. Throughout the post-operative period of five months, the testes were known to be in the scrotum. There were no signs of degeneration in the prepared slides of either testis.

Rat 16 no. 42. Age, forty days when bilateral vasectomy was performed. The animal was killed four months later, and during this time the testes were known to be in the scrotum. Microscopically, both testes were normal.

Rat 42 no. 55. Adult. Ligation of the vasa deferentia was for one month. The testes were in the scrotum during this period, and both were normal when the animal was killed.

In this group of six animals, the testes were known to be in the scrotum from the date of operation until killed. In rats one can usually determine the presence of the testes in the scrotum by observation, but in cases of doubt these animals were palpated. In guinea pigs palpation was always resorted to in order that there might be no uncertainty. In none of these cases was there found any indication of changes in the testes from the operation. The microscopic picture of each testis was normal.

B. In order to determine the full significance of these findings another series of operations was tried. In this series the testes were known to be in the scrotum following the operation. Thirty to sixty days later one testis was pulled from the scrotum into the abdominal cavity and there made fast to the belly wall. Four such experiments are here given:

Rat 43 no. 56. Adult. The vasa deferentia were ligated and resected. The testes were ascertained, by palpation, to be in the scrotum during a period of thirty days following the operation. A second operation was then performed in which the right testis, then normal in size, was pulled from the scrotum into the abdominal cavity and lightly stitched to the abdominal wall by passing a silk thread through the fat-body of the testis and through the peritoneum and an abdominal muscle. During the fifteen days following, the left testis remained in the scrotum, the right testis in the abdominal cavity. The animal was then killed; both vasa deferentia were closed by the ligatures and the testes were noted to be in the locations desired in the experiment. It was noted that the right testis was not more than one-half its former size as seen at the time it was fastened in the body cavity or of that of the left testis. When microscopic examinations were made it was found that the left testis was normal and full spermatogenesis was in progress. In the right testis, on the other hand, the germinal epithelium had almost completely degenerated. This had happened presumably in fifteen days.

Rat 45 no. 58. Adult. This animal was operated upon in the same manner as the preceding with the exception that the left testis, then normal in size was fastened to the abdominal wall at the end of the first thirty days instead of the right testis. When the animal was killed fifteen days later (forty-five days after the initial operation) the

ligatures on both vasa deferentia were firm. The left testis was no more than one-half its former size as noted at the time it was fastened to the abdominal wall or of the right testis. Microscopic observations revealed the fact that the right testis was normal, while the seminiferous tubules of the left testis were almost devoid of spermatogenic content.

Rats 44 no. 57 and 46 no. 59, both adults, were operated upon in the same manner as those just described. In these two cases, however, thirty days elapsed between the date of the second operation and that of killing. The results were similar, except that degeneration was here more complete in the testes retained in the abdominal cavity. The testes that remained in the scrotum following vasectomy were normal.

C. The results of the above four experiments seemed conclusive, but in order that there might be no uncertainty in the matter another series of experiments was prepared.

Four rats were used: 29 no. 32, 30 no. 39, 31 no. 49 and 28 no. 52, the first three were four and one-half months old, the last four months old. Both vasa deferentia were ligated and resected in each animal; for a period of three months after the operation the testes were ascertained by palpation to be in the scrotal sacs. In a second operation made three months after the first, the left testes, then normal in size, were pulled into the abdominal cavity and there made fast to the belly wall as in former experiments. These animals were killed fifteen days later, three and one-half months after the initial operation. The ligatures were all firm and in each case the left testis was about one-half its former size as noted at the time of the second operation or of that of the right testis. In every experiment the right testis was normal in microscopical structure while the left testis was almost devoid of its germinal content. This condition is shown very well by the illustrations, figures 3 and 4, showing representative portions of the right and the left testis of rat 30 no. 39 of this series. From these experiments it is very evident that ligation of the vas deferens does not of itself cause degeneration, but when the testis, after ligation of the vas deferens, is retained in the abdominal cavity, degeneration always follows very rapidly.

In order to further determine the relative importance of these two factors (ligation of the vas deferens and retention of the testis in the abdominal cavity) as causes of the changes produced in the testis a crucial experiment was devised.

Rat 38 no. 36. Adult. After the laparotomy the right vas deferens was ligated and resected without removing the testis from the scrotum. The left testis was then carefully pulled from the scrotum and fastened in the abdominal cavity without in any way injuring the vas deferens of that side. As in former experiments the thread fastening the testis to the belly wall passed through only fatty and connective tissues of the testis and in no way injured the tunica albuginea or the content of the testis. The rat was killed one month later at which time the ligature on the right vas deferens was still firm and the left testis, now no more than one-half the size of the right testis, was retained in the abdominal cavity. When microscopic preparations were examined

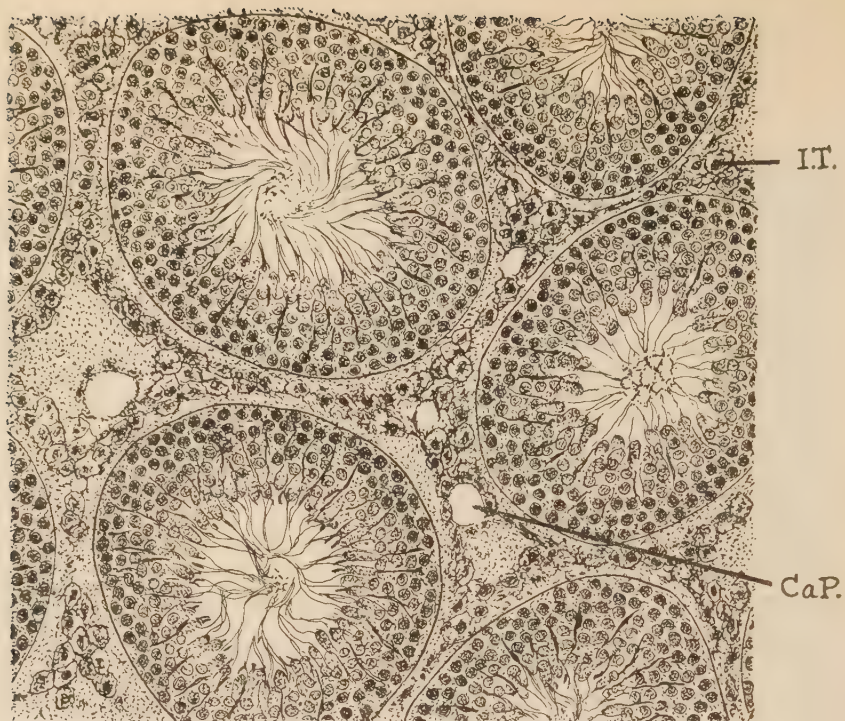


Fig. 3. Portion of right testis of rat 30 no. 39. *IT*, intertubular tissue; *Cap*, capillary.

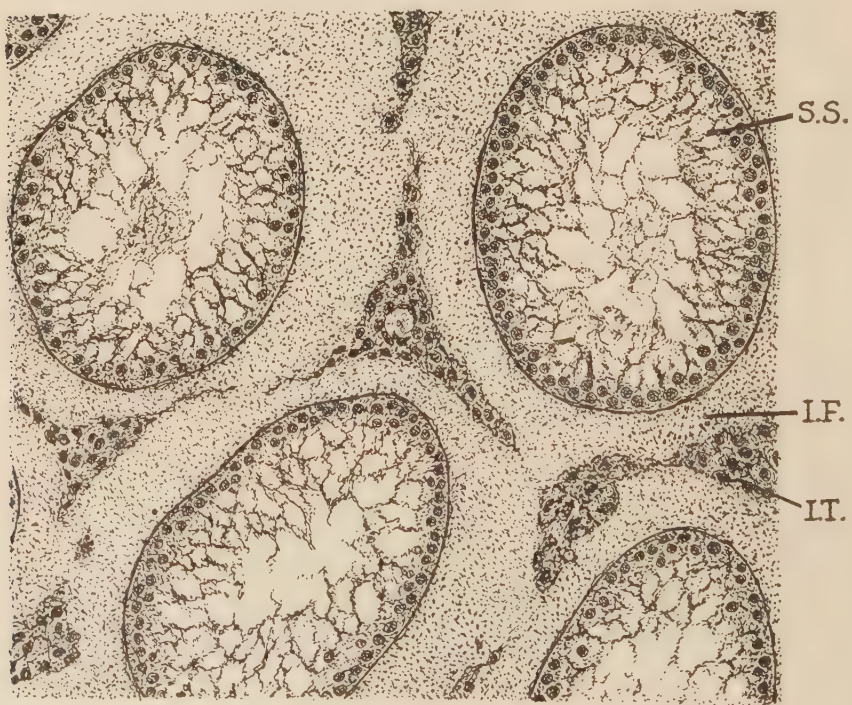


Fig. 4. Portion of left testis of rat 30 no. 39. *IF*, intertubular fluid; *IT*, intertubular tissue; *SS*, syncytium of supporting epithelial cells.

it was found that the right testis (with ligated vas deferens) was normal, while the left testis which had been retained in the abdominal cavity but with vas deferens uninjured, had undergone extensive degeneration. The seminiferous tubules were almost empty and there was some hypertrophy of the intertubular tissues. Figures 1 and 2 represent typical tubules of the right and left testes of this animal, and show in a striking manner the relative importance of retention of the testes in the abdominal cavity in contrast with ligation of the vas deferens.

Rat 54 no. 28. Adult. Both testes, without injury to the vasa deferentia, were fastened within the abdominal cavity. Four months later when the animal was killed it was found that the left testis had become free and returned to the scrotum. Microscopic examination showed that both testes had undergone degeneration, but that the left testis was more nearly normal than the right testis. It was inferred that the testis upon returning to the scrotum had partially recovered from the degenerative changes undergone while retained in the abdominal cavity. The interstitial tissue between the tubules seemed packed more closely among those tubules that contained cells undergoing active spermatogenesis. Between neighboring tubules, where spermatogonia were abundant but where only a few spermatocytes were seen, the interstitial tissue appeared more loose and more nearly like that of the right testis. Division figures in the spermatogenic cells appeared more abundant than in the normal testes and the inference that regeneration took place in this testis upon returning to the scrotum therefore seems justified.

This experiment shows that retention of the testis in the abdominal cavity produces degeneration of the germinal epithelium, and that this (artificial cryptorchidism) has been an influence in the above experiments where degeneration has been produced. Sand (1921) has described similar experiments where one vas deferens only was ligated and the contralateral testis, with vas deferens intact, was fastened in the abdominal cavity. On page 522, table III, he gives the results of three experiments of this nature. Sand was interested in the interstitial cell phenomena and made no comment upon the material from the germinal tissue viewpoint, other than to record the general findings. An analysis of his experiments and their results indicates that ligation of the vas deferens (in these cases, nine months, nine months nine days, and five months respectively) has not caused atrophy of the tubular content. Retention of the testis (vas deferens not ligated) has in each case caused atrophy of the tubular content and hypertrophy of the interstitial tissue.

Partial castration. In view of the fact that very little hypertrophy of the interstitial tissue resulted in my experiments a series designated as "partial-castration experiments" was tried. In these experiments only a small portion of one testis was left in each animal. The technique used was the same as that employed by Lipschütz (1922). This series consists of eleven experiments, ten of which agree in general with the results described by the above worker, i.e., in complete degeneration, resorption or in cyst-formation of the remaining piece. My experiments do not, however, admit of as simple an explanation as Lipschütz gives for his series. Certainly the results cannot be interpreted in terms

of blood supply alone as Lipschütz supposes. Guinea pig 47 no. 41 of this series lived three months after the operation. When killed, the fragment left in the animal was found in the scrotum and, when sectioned, the tubules were found to be full of spermatogenetic tissue and to all appearances normal. In the remaining ten cases the testis fragments were found in the abdominal cavity and devoid of germinal epithelium. Though there are several factors present complicating the results of these experiments, the fact that germinal epithelium was present in the one case where the fragment was located in the scrotum supports the thesis that location of the testis is here a prime factor as was found in vasectomy experiments.

Throughout these experiments the interstitial cell phenomena have been studied, but the influencing conditions have been so numerous that a solution of this phase of the problem has not been attained. Very little hypertrophy of interstitial tissue and certainly no compensatory hypertrophy was found. A full discussion of the influencing factors and relationships found in these experiments has been reserved for a later paper.

Discussion. It seems conclusively proved that degeneration of the germinal epithelium results from displacement of the testis from the scrotum to the abdominal cavity and that vasectomy alone does not cause degenerative changes in the testis. In every experiment in which the testis was retained in the abdominal cavity, the spermatogenetic tissue underwent degeneration. In some of the experiments the vas deferens had been ligated before the testis was fastened in the abdominal cavity, but in the last two experiments, given above, and in the series by Sand, above cited, the testes were placed in the abdominal cavity without injury to the vas deferens. Degeneration had in each case resulted from displacement of the testis. On the other hand, degeneration of the germinal epithelium has not followed ligation of the vas deferens in any experiment where the testes were known to reside in the scrotum following the operation. In many of the experiments given above and in many of those not here included, ligation of the vas deferens had been for a period of ten months without any degenerative effect upon the testes. The size of the testis, the size of the attached fat-body and the size and shape of the inguinal canal, are all factors here involved.

The above explanation satisfies all the conditions met with in these experiments and all thus far met with in the literature where sufficient data are given to make an interpretation possible. In the postulate that degeneration of the germinal epithelium is a function of the location of the testis, the conditions met with in all cases of vasectomy are satisfied.

Other views have been presented by writers to explain the variable results. Ancel and Bouin have stated that degeneration always resulted

if the experiment was continued long enough, but in many of my experiments no change took place in the testis even after ligation and resection of the vas deferens for a period of six to ten months. It would seem that at least one-third the span of the animal's life would be ample time for the appearance of the changes, especially since degeneration has been found in many cases within fifteen days, when the testes were fastened in the abdominal cavity.

Sand (1921), while calling attention to the irregularities in his experiments, explains them by saying that while degeneration takes place quickly in adults, it does not commence until after puberty. On page 499 (1922) he presents a table showing the results of operations on two rats and two guinea pigs. In his summary he states that in four cases of bilateral vasectomy performed on young animals no distinct effect was produced after five to twelve months and only in one case (M. 114) was there any effect and in that experiment beginning after the twelfth month. It is the youngest animal in his series that shows the degeneration and it is probable that the testes in the other case were normal because they returned to the scrotum. On page 520 he gives another table showing that the testis of a rat twenty-one days of age (C. 154) when retained in the abdominal cavity underwent degeneration. In his series, cited to show that degeneration does not take place in young animals, a number of cases three months of age are included. Lipschütz (1922) on page 133, says, "Sand showed that the remaining testicle can undergo the same hypertrophy even when the vas deferens is ligatured; this is due to the fact that, even after the vas deferens is ligatured, the spermatogenesis can proceed in a normal way, and the degeneration of the tubules begins only when the spermatogenesis is more or less completed."

The above authors do not state their criteria for "young animals" but the writer finds that spermatogenesis is in progress and spermatozoa present in rats and guinea pigs before they are three months of age and extensive degeneration was found following vasectomy in a rat (15 no. 41) only forty days old.

Tiedje (1921) found that when one testis of young guinea pigs is removed and vasectomy performed on the other side, the remaining testis develops normally, but that when such operations are performed on older animals, there is at first degeneration followed by complete regeneration. He performed the operations by laparotomy and the writer's experience indicates that in young animals the testes readily return to their normal position in the scrotum, while in adult animals, due to factors to which attention had already been called, they often remain in the abdominal cavity after such operations.

Of eleven experiments by Tiedje, eight of the animals showed, according to his interpretation regeneration after degeneration, while three

remained in the degenerated condition. He offers no data to prove his assumption that degeneration actually took place, and was then followed by regeneration. In the eight experiments, Tiedje ligated and resected the vas deferens distal to the epididymis. In these there was least likelihood of retention of the testes in the abdominal cavity, which accounts for their non-degenerated condition. In the other three cases he ligated and resected between the epididymis and the testis which involves a greater amount of handling of parts with a greater probability of trauma and adhesions within the abdominal cavity. This would account for their degenerated condition.

The assumption of degeneration with subsequent regeneration is also made by Steinach (1921) in his experiments on rats and guinea pigs. Like Tiedje, however, he gives no data to prove that this is the usual course of events, but the conclusion is derived from comparisons of cases where degeneration was found in the testes of animals killed shortly after vasectomy with others in which no degeneration was found in testes of animals that had lived for a longer time following vasectomy. In his operations he not only pulled the testes into the abdominal cavity, but while examining them he pulled them out into the open. He then ligated the vasa deferentia and returned the testes to "their former site." The more the testes are handled, the more likely is it that trauma will take place and adhesions form, which would account for the cases of degeneration. But it is also a common occurrence for testes to return to the scrotum and thus the cases in which there is no degeneration are readily explained.

Myers (1915) reported on a series of rats subjected to vasectomy by Stotsenberg in which he describes degeneration of increasing completeness the longer the animal had lived following the operation. No regeneration was found in that series.

If occlusion of the duct is sufficient to cause degeneration it does not seem reasonable to assume that regeneration can take place until the causal factor has been removed, even if the experimental analyses were ignored. The writer has been unable to arrange his experiments in any order that would bear out the contentions of Steinach or Tiedje that degeneration followed by regeneration results from vasectomy. Degeneration is present in experiments of ten months' duration as well as in those of fifteen days.

In seeking an explanation other than that of juvenile state to account for those cases where degeneration did not follow ligation of the vas deferens, Sand (1921) noted that cysts or pocket-like structures often formed at the resected end of the vas deferens. On page 503 he says that he is inclined toward the opinion of Tournade, that the cysts have an importance in the results following resection, but he does not un-

equivocally align himself with that viewpoint. As Sand states, these cysts are not constantly found and their importance in explaining the absence of degeneration is for this reason doubtful. Moreover, in my series of experiments, these cysts have been found fifteen days after operation in cases in which degeneration of the germinal epithelium has nevertheless occurred. In this connection it seems worthy of mention that in many experiments of both short and long duration the tubules of the epididymis were large and distended. Sections of such tubules showed them to be full of normal and degenerating spermatozoa. Sections of the cysts showed them to be full of clumps or nodules of what appeared to be agglutinated spermatogenic cells, but there is no correlation between cyst formation and the condition of the germinal epithelium.

Discussion and comparisons have thus far been limited to rodents. In these animals, at least all those used in this type of experiment, the inguinal canal is open and the testes are free to pass in and out of the abdominal cavity. It is further seen that in all cases when the testes are retained in the abdominal cavity for even a short period (fifteen days) degeneration takes place.

A few workers have used carnivora and with the exception of Kuntz (1921) they report that no changes are found following vasectomy. Shattock and Seligman (1904), working on sheep, state on page 51 that "when the vas is cleanly isolated and divided after ligation without the inclusion of vessels, or without the subsequent occurrence of thrombosis, no atrophy of the gland ensues." The same condition has been found in man, according to all reliable reports, where the vas deferens has been occluded by accident or by disease. In these three orders of mammals the testes are contained in a pendulous scrotum with the inguinal canal closed, and the location of the testes is known to be in the scrotum. The results are therefore not confused by artificial cryptorchidism as when rodents are used. Cooper (1827), using dogs; Curling (1843), using cats and dogs; Gosselin (1853), using dogs; Guyon (1895), using dogs; Wheelon (1921), using dogs; and Wallace (1904), using dogs and cats, have all found that degeneration does not follow ligation of the vas deferens. From a few months to four years elapsed between the date of operation and the date when the animals were killed. In the one series of experiments on carnivora where degeneration was reported as a result of vasectomy the author, Kuntz (1921, p. 198) says of his controls: "One unoperated dog, kept for one month with some of the operated dogs as a control animal, showed degenerative changes in both testes. Another dog was subjected, under ether anesthesia, to an operation not involving the genital organs and kept under essentially the same conditions as the other experimental animals for four months following the operation, when both testes were removed and prepared for study. Preparations of both testes in this

case showed advanced degenerative changes in the seminal epithelium; however, spermatogenesis was not entirely suspended." When the control and experimental animals show like abnormal conditions, no changes can be ascribed as resulting from operative procedures.

The medical literature contains many cases that leave no doubt that no changes occur in the human testis from closure of the vas deferens. A few examples will suffice. Spangaro (1904) found spermatozoa in the testes of two men on whom vasectomy had been performed six months and two and one-half years respectively. Simmonds (1921) examined forty human cases in which one or both vasa deferentia had been closed by cicatrix formation. In thirty cases spermatogenesis was normal. Case histories are not known for the ten cases not accounted for and complications may have been present. Some years ago vasectomy was practised as a substitute for castration for the relief of enlarged prostate gland. It soon became evident that spermatogenesis was not hindered and the relief was not forthcoming while the testis was present. The literature is too voluminous for review here but the great number of such cases reported in that field offers strong evidence that degenerative changes do not result from vasectomy in man. It is therefore evident that in those animals where the testes are suspended in a pedunculate scrotum, where the results are not confused with artificial cryptorchidism, the testes are unaffected by vasectomy. The ligation periods have been from a few months to years and are reported by so many workers that the evidence is unquestionable.

In striking contrast to the results obtained from this type of operation upon these animals, are the results found in cases of cryptorchidism. Among the early workers reporting on such material were Curling (1843), Cooper (1827), Goubaux and Follin (1855), Godard (1856), Monod and Arthaud (1887) and Griffiths (1893). They all agree that cryptorchid testes are devoid of spermatozoa. The material used by these workers varied widely—pigs, horses, dogs and man being included. During recent years many workers have affirmed these findings, among them: Hanes (1911), reporting on pigs, sheep and man; Loeb (1918), on guinea pigs; Goddard (1920), on man, and Moore (1923) on rats. Goddard states (p. 175): "In all the retained organs examined, there was complete atrophy of the contents of the seminiferous tubules." Moore's descriptions of cryptorchid testes fit conditions illustrated in figures 2 and 4 here given. Ancel and Bouin (1903) describing the appearance of the degenerative conditions found by them in testes following vasectomy called attention to the fact that the results resembled in all respects those found in cryptorchid testes. There is every reason to believe that the resemblance was more complete than they supposed. The results they were describing were doubtless those of artificial cryptorchidism and not of vasectomy.

It is the writer's belief that these findings will aid in the interpretation of experiments involving transplantation of ovaries and testes. Ovaries, when used as grafts, generally grow and produce normal germ cells. This fact has made possible the studies by Guthrie (1909, 1911), and by Castle and Phillips (1911), in which the effect of the environment upon the ovum and embryo in guinea pigs was studied. Moore (1921) showed that ovaries transplanted into male animals will grow and produce germ cells for at least eight and one-half months. Grafts of testes, on the other hand, do not produce spermatozoa in the majority of experiments. Though the grafts persist, the seminiferous tubules are empty or contain only spermatogonia. The grafts have usually been placed intraperitoneally, intramuscularly or subcutaneously. If normal, uninjured testes degenerate when placed in the abdominal cavity, one cannot reasonably expect portions of testes to grow in this environment. Those grafts placed subcutaneously should be the most likely to produce normal spermatogenetic cells, since this position is most nearly like that of the scrotum which is a pouch composed of skin and the dartos tunic, and it is these grafts that are most frequently figured in the various papers dealing with testes grafts. That the site of such implantations is important has been shown by Moore (1923), who has found spermatozoa in grafts of rat testes placed in the scrotum.

In birds the anatomical and physiological conditions are altogether different from those in mammals and yet are easily understood from the point of view of location of the testis. Shattock and Seligman (1904) and Massaglia (1920) have shown that ligation of the vas deferens in cocks does not cause degeneration of the germinal epithelium. The former writers found this in all cases while the latter is not so definite and states that the results are not always the same. In birds not only do grafts of ovaries grow, but pieces of testis grafted on the peritoneum grow readily and produce normal appearing spermatozoa. This has been shown by Shattuck and Seligman (1904), Guthrie (1910), Massaglia (1920) and Goodale (1916). The natural environment of both the ovary and the testis in birds is in the abdominal cavity, and when vascularization is not hindered both vasectomized and grafted testes grow and produce spermatozoa.

In brief, it has been shown by various workers that grafts of ovaries of birds and mammals grow readily and produce functional germ cells; that testis grafts in birds likewise produce normal appearing germ cells while testis grafts in mammals do not produce spermatozoa when placed within the abdominal cavity, but do so when they are placed within the scrotum (Moore, 1923). It has also been shown that vasectomy is not followed by degenerative changes in the testis when performed upon birds or upon mammals where the testes reside in a pendulous scrotum. It

has also been shown that in the hands of many experimenters it often happens that no changes are found to follow vasectomy performed upon rats, guinea pigs, and rabbits—Gosselin (1853), Brissaud (1880), Sand (1921) and Lipschütz (1922). Since former workers have failed to note the location of the testes in the ligation experiments and since, in these new experiments, the location of the testes has been controlled, there can be no doubt that the explanation offered is the correct one.

It is well known that the germinal epithelium of the testis is very sensitive to other factors than here described. Paul (1906) found that in rats fed on an exclusive meat diet the reproductive organs were much less developed than in controls. Houssay (1907) and Pezard have conducted similar experiments upon birds, each finding effects leading to progressive sterilization and, according to the former, to loss of sexual dimorphism. It is interesting to note that fattening of the male domestic goose modifies the secondary sexual characters and interferes with the onset of the breeding period (Steive, 1921). Acute alcoholism produces similar results. Bertholet (1909), Wiechelbaum and Kyrle (1912), MacDowell and Vicari (1917), Allen (1919 b), and Kostitch (1921) have all reported degenerative effects upon the germinal epithelium from the use of alcohol. Kostitch examined the testes of many men who died of acute alcoholism and stated (p. 675) that in an advanced state, the tubules appear only as a syncytium, without the layers of spermatogenic cells and consist of no more than a single row of sertoli nuclei. Cordes (1898) reported an aspermatic condition in twenty autopsies of men between the ages of twenty-one and sixty who had suffered but a short time from pneumonia. Wollback (1919) has published similar findings in cases where death was due to influenza. Jacobson (1898) noted that an increase of interstitial cells was associated with inflammation and this is likewise known to be true in orchitis from mumps. In addition to the above some work has been done that indicates that feeding of thyroid interferes with the normal development of the sex organs and the secondary sexual characters (Gudernatsch, 1915; Horning and Torrey, 1922). Meat diet, obesity, alcoholism, fever and inflammation, and possibly thyroid feeding, all produce changes in the testes that closely resemble those found in cryptorchidism.

The evident factor common to all the above conditions is a rise in temperature which is either local or general. In the case of orchitis, there is a local inflammation with accompanying rise in temperature. In obesity, the accumulation of fat in the cutaneous part of the scrotum is sufficient to cause a rise in temperature within. In meat diet, alcoholism, fever and thyroid feeding there is a general rise in temperature of the body which affects the testis, whether its normal residence is in the body cavity, as in birds, or in a scrotum. Where the rise in temperature is general

throughout the body it should affect the ovary as well as the testis and this has been found to be the cause, experimentally, in thyroid feeding and in acute alcoholism. The temperature within the abdominal cavity is appreciably higher than that within the scrotum and it is probable that this is a factor causing degeneration of the germinal epithelium. That this difference of temperature exists has been indicated by the work of Benedict and Slack (1902), showing a temperature gradient of about 5°C . between that of the abdominal cavity and that of the surface of the body. Hart (1922) kept mice in large glass chambers at abnormal temperatures of 32° to 40°C . and at 4° to 7°C . for periods of varying numbers of days in an attempt, by experimental means, to analyze the effect of temperature on the thyroid and testes. In a description of results from one experiment he says (p. 166) that in testis (illustration 5) the spermatogenesis is completely ended and presents a picture of extreme degeneration with the majority of canals having only a single layer of sertoli cells left. That temperature is here the specific influencing factor cannot be held since changes in other glands, especially that of the thyroid, may have and very probably did have an important part in the results found in the testes. The experiments with thyroid feeding attest to this probability. Hart's experiments indicate the importance of temperature as an influencing factor though they do not show it to be the direct effect causing degeneration of the germinal epithelium. Crew (1922) has postulated high temperature as a cause of the aspermatic condition of undescended testes but offers no experimental data in support of his contentions.

SUMMARY

1. There is much disagreement among experimenters as to the effect of vasectomy upon the testes. The early workers have failed to take into account a variable factor in their experiments.
2. It was found that in all experiments where the testes resided in the scrotum following vasectomy no degeneration has taken place.
3. In all experiments in which the testes were retained in the abdominal cavity with or without vasectomy, degeneration took place.
4. In those mammals where the testes are contained in a pendulous scrotum so that the results are not complicated by artificial cryptorchidism, vasectomy has not produced degeneration.
5. In birds where the position of the testes is not disturbed in performing the operation, vasectomy does not produce degeneration.
6. Testis grafts in mammals do not produce spermatozoa when placed in the abdominal cavity if their normal residence is in a scrotum. Such grafts produce spermatozoa when placed in the scrotum.
7. Vasectomy, as such, does not cause degeneration of the germinal epithelium. This has been shown in experiments of ten months' duration.

8. It is suggested that degeneration of the germinal epithelium is caused by a rise in temperature which may be either local or general in the body of the animal affected.

BIBLIOGRAPHY

- ALLEN. 1919a. *Anat. Record*, xvi, 25.
 1919b. *Anat. Record*, xvi, 93.
 ANCEL AND BOUIN. 1903. *Arch. d. Zoöl. Exper. Series 4*, vol. i, 437.
 BALTZER. 1912. *Mittl. Zoöl. St. Neapel*, 22.
 BENEDICT AND SLACK. 1911. *Carnegie Publication 155*.
 BERTHOLET. 1909. *Centrabl. f. allg. Path. u. path. Anat.*, xx, 1062.
 BRISSAUD. 1909. *Arch. d. Physiol.* 2nd series, vii, 769.
 CASTLE AND PHILLIPS. 1911. *Carnegie Inst. Wash. Publ. no. 49*.
 COOPER. 1827. *The anatomy and surgical treatment of abdominal hernia*. London, 1827.
 CORDES. 1898. *Virchow's Arch. path. Anat.*, cli, 402.
 CREW. 1922. *Journ. Anat. and Physiol.*, lvi, 98.
 CURLING. 1843. *A practical treatise on the diseases of the testis, and of the spermatic cord and scrotum*. Philadelphia, 1843.
 EVANS AND BISHOP. 1922. *Journ. Metabolic Research*, i, 335.
 GODARD. 1856. *Compt. rend. Soc. de Biol.*, 2d series, 111.
 GODDARD. 1920. *Journ. Anat.*, (London) liv, 173.
 GOLDSCHMIDT. 1917. *Endocrinol.*, i, 433.
 GOODALE. 1916. *Carnegie Inst. of Wash. Publ. no. 243*.
 GOSSELIN. 1853. *Arch. gen. de Med.*, Paris 5. s. 11.
 GOUBEAUX ET FOLLIN. 1855. *Mem. d. la Soc. de Biol.*, 1855.
 GRIFFITHS. 1893. *Journ. Anat. and Physiol.*, xxvii, 483.
 1894. *Journ. Anat. and Physiol.*, xxiv, 209.
 GUDERNATSCH. 1915. *Anat. Record*, xii, 78.
 GUTHRIE. 1909. *Science N.S.*, xxx, 724.
 1910. *Journ. Exper. Med.*, xii, 269.
 1911. *Science N.S.*, xxxiii.
 GUYON. 1895. *Lecons cliniques sur les affections chirurgicales de la vesie prostate professées a l'Hospital, Necker, recueillies et publiees par F. D. Guiard VII*, 9th. *Surgical Congress*, 1888.
 HANES. 1911. *Journ. Exper. Med.*, xiii, 338.
 HART. 1922. *Pflüger's Arch.*, cxcvi, 151.
 HORNING AND TORREY. 1922. *Anat. Record*, xxv (abstract).
 HOUSSAY. 1907. *Arch. d. Zoöl. exper. et gen. Hist. Nat.*, 4s. 6, 137.
 JACOBSON. 1879. *Arch. f. path. Anat.*, lxxv.
 KOSTITCH. 1921. *Compt. rend. Soc. de Biol.*, lxxxiv, 674.
 KUNTZ. 1921. *Endocrinol.*, v, 190.
 LOEB. 1918. *Biol. Bull.*, xxxiv, 33.
 LIPSCHUTZ, OTTOW AND WAGNER. 1922. *Proc. Royal Society*, xciii, 132.
 MACDOWELL AND VICARI. 1917. *Proc. Nat. Acad. Sciences*, iii, no. 9.
 MASSAGLIA. 1920. *Endocrinol.*, iv, 547.
 MILLS. 1919. *Journ. Exper. Med.*, xxx, 505.
 MONOD ET ARTHAUD. 1887. *Arch. Gen. de Med.*, 1887.
 MOORE. 1921. *Journ. Exper. Zoöl.*, xxxiii, 129.
 1923a. *Anat. Record*, xxv, 142.
 1923b. *Science* (in press).

- MYERS. 1915. Anat. Record, x, 228.
- PAUL. 1906. Proc. Physiol. Soc. London, Journ. Physiol., 14.
- PEZARD. 1919. Compt. rend. Acad. d. Sciences, clxix, 1177.
- RIDDLE. 1917. Science, N.S., xl, 19.
- SAND. 1921. Journ. Physiol., xix, 305, 494, 515.
- SHATTOCK AND SELIGMAN. 1904. Proc. Royal Soc. of London, lxxiii, 49.
- SIMMONDS. 1921. Deutsch. Med. Wochenschr., xlvii, 665.
- SMITH. 1910. Quart. Journ. Micros. Sci., liv, 577.
- SPANGARO. 1904 and 1905. Rev. veneta di sci. med., xlii, 21, 80, 140, 201, 255.
Lo Spermintale an 57, F. 3, Brit. Med. Journ.
- STEINACH. 1921. Arch. f. Entwickmech., xlvi, 557.
- STIEVE. 1921. Anat. Anz., liv, 63.
- TIEDJE. 1921. Deutsch. Med. Wochenschr., xlvii, 352.
- WALLACE. 1904. Trans. Path. Soc. of London, lvi, 80.
- WEICHELBAUM AND KYRLE. 1912. Sitzungsab. k. akad. Wissensch. Med. naturw. Kl., cxxi, 51.
- WHEELON. 1921. Endocrinol., v, 307.
- WOLBACH. 1919. Johns Hopkins Hosp Bull., xxx, 104.

